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Pleistocene speciation is not refuge speciation

ABSTRACT

A number of researchers working on the origin of extant Neotropical biodiversity implicitly and without appropriate proofs assume that Pleistocene speciation should necessarily follow the rules of the refuge hypothesis. A recent example is provided by a study of Neotropical butterflies. Although the analysis showed that these groups experienced their main diversification burst during the last 2.6 million years, coinciding with the Pleistocene glacial cycles (Garzón-Orduña *et al.*, 2014, *Journal of Biogeography*, **41**, 1631–1638), a causal link between the speciation chronology and the evolutionary mechanisms proposed by the refuge hypothesis is not provided. Without more detailed studies on the environmental drivers, geographical patterns and speciation modes, establishing a causal link between speciation chronology and a particular speciation model – of which the refuge hypothesis is only one among many possibilities – is too speculative. Here I provide a six-step conceptual framework for linking the speciation chronology with the environmental drivers and the ecological and evolutionary mechanisms potentially involved.

Keywords Butterflies, climate change, diversification, Neotropics, Pleistocene, refuge hypothesis, refugia theory, speciation.

Garzón-Orduña *et al.* (2014) used DNA molecular phylogenies to estimate the age of speciation of Neotropical butterflies in their contribution to the debate about the timing and causes of Neotropical diversification. These authors addressed the problem using species dating (estimating the age of divergence of sister species) instead of crown dating (estimating the age of the initial diversification of supra-specific clades), as it has been suggested that, in general, crown dating tends to overestimate the chronological origin of diversity (Rull, 2011a,b). Garzón-Orduña *et al.* (2014, p. 1631) found that ‘...72% of speciation events leading to the formation of butterfly sister species occurred within the last 2.6 Myr [the Pleistocene]...’ and concluded that ‘...the PRH [Pleistocene refuge hypothesis] cannot be completely discarded as a driver of Neotropical diversification’. In their analysis, Garzón-Orduña *et al.* (2014) were able to overcome the problems associated with crown dating, which prevents a sound assessment of the origin of Neotropical diversity. However, these authors failed to circumvent another paradigm that is still firmly embedded in the minds of some researchers: the idea that Pleistocene speciation should necessarily follow the propositions of the refuge hypothesis. Here, I will discuss this issue from a conceptual perspective.

In a recent paper (Rull, 2013), I analysed the three main problems that, in my opinion, are slowing progress towards understanding of the origin of Neotropical diversity. The problem of taxonomic resolution (species-dating versus crown-dating inferences) and the danger of over-generalizations from one or a few case studies or from geographical settings are satisfactorily addressed by the work of Garzón-Orduña *et al.* (2014) and others. However, other paradigms still survive. Placed in the correct context, the results of Garzón-Orduña *et al.* (2014) could be extremely useful to circumvent the so-called

‘paradigm shifting’ handicap; that is, the replacement of an unproved paradigm by another equally useless paradigm (Rull, 2013). In the present context, the old paradigm was the refuge hypothesis, stating that most extant Neotropical species originated during the Pleistocene in response to successive glacial contractions (vicariance), interglacial expansions and coalescence (gene flow) between populations in lowland Amazon rain forests. Support for the refuge hypothesis was based primarily on present-day biogeographical patterns and palaeoecological reconstructions (Whitmore & Prance, 1987), yet new evidence (e.g. Colinvaux *et al.*, 2000) has called this hypothesis into question. Further developments, derived from DNA molecular phylogenetics using crown dating, provided mostly pre-Pleistocene diversification ages for Neotropical clades and suggested a new paradigm that Neotropical diversity originated before the Pleistocene, most likely during the Miocene, mainly as a consequence of Andean orogeny and the ensuing palaeogeographic re-arrangements (Hoorn *et al.*, 2010).

The defenders of this new paradigm claim that the Pleistocene should no longer be considered a time of diversification in Amazonia. Rather, ‘the Quaternary should possibly be considered as a period of net loss of biodiversity’ (Wesselingh *et al.*, 2010, p. 424). These arguments were also used to dismiss the refuge hypothesis as a valid speciation model for the Neotropics on the basis of merely chronological reasons by equating Pleistocene speciation with refuge speciation. In this way, these two concepts became intrinsically linked in the minds of many researchers as a derived paradigm, and for these researchers, the debate turned into a dual dispute between the defenders of the Miocene-orogenic hypothesis and the Pleistocene-refugial hypothesis. Garzón-Orduña *et al.* (2014) could have used their results to challenge this simplistic ‘paradigm shifting’ framework, but instead, these authors came back to the former Pleistocene-

refugial paradigm. Previous literature reveals that the refuge hypothesis is only one of many speciation models proposed for the Neotropics (and the tropics, in general) and is not always linked to Pleistocene glacial cycles. Other models include the riverine barrier hypothesis, the gradient hypothesis, the canopy density hypothesis, the biotic interaction hypothesis or the disturbance-vicariance hypothesis, to name a few (see Nores, 1999, and Haffer, 2008, for a comprehensive account). Therefore, demonstrating Pleistocene speciation is not the same as supporting the refuge hypothesis.

At least six conceptual issues should be considered when dealing with the origin of Neotropical biodiversity using palaeoecological and molecular phylogenetic evidence (Fig. 1). The first step is (1) to clearly set the research question to be addressed and to collect or select the empirical data needed to test the corresponding hypotheses. This is a crucial step as it constrains all the ensuing research and expectations. In the next steps, (2) empirical data provide information on speciation timing, which (3) can be used to detect chronological correlations with known environmental shifts that might drive speciation. It is important that this third step is conducted in a collaborative, multidisciplinary manner to avoid circularity (Baker *et al.*, 2014). Once this information has been gathered, the next steps are (4) to infer the speciation modes linked to (5) the geographical reorganizations resulting from the candidate environmental shifts. A classic example is the closure of the Panama Isthmus (Pliocene), leading to allopatric speciation in the Caribbean and the Atlantic, as well as gene flow and adaptive radiations between North and South America. Finally, speciation is not an instantaneous event but the result of interacting ecological and microevolutionary mechanisms and, therefore, (6) the particular processes involved for each species and the communities to which the species belong should be properly addressed for a complete understanding of

a given system. For example, environmental heterogeneity is an ecological feature that is sometimes forgotten but is of paramount importance for the origin and maintenance of endemism and therefore biodiversity (Stein *et al.*, 2014). In this sense, it should be stressed that the Neotropical Amazon basin, for instance, is as large as Europe and exhibits exceptional spatial heterogeneity.

Each of these conceptual steps requires its own research plan that is associated with pertinent hypotheses and suitable empirical data to test them. Additionally, the hierarchical chain of reasoning cannot be broken; for example, geographical patterns and/or speciation modes cannot be deduced in a straightforward fashion from evidence merely of timing of events. In the case of Neotropical butterflies, Garzón-Orduña *et al.* (2014) made a conceptual leap from step (2) to (5) without the necessary evidence. Pleistocene speciation is nicely supported, but a discussion on the environmental drivers involved, the probable speciation modes and the likely relevant geographical patterns is not provided. Therefore, the main conclusion of this work may have been that Pleistocene speciation was dominant in these butterflies, highlighting the potential role of ecological and evolutionary mechanisms linked to climate changes (indeed, the major Pleistocene environmental disruption was caused by glacial–interglacial cycles and associated sea-level changes) (Flint, 1971; Bradley, 1999). To progress from this point to more detailed explanations requires a research plan that includes hypotheses on speciation modes, geographical patterns and ecological mechanisms as well as a description of the empirical data needed to test such hypotheses. This does not mean that all six points depicted in Fig. 1 should be addressed in each single study or by each research team but they illustrate the conceptual complexity of this type of research, in

which biogeographical, ecological, geological, evolutionary and environmental elements, among others, constantly interact through time and across space.

Studies such as those of Garzón-Orduña *et al.* (2014) should be encouraged, as they are essential for disentangling the complexity of Neotropical diversification patterns in time and space, but they should be placed in the appropriate conceptual context of Chamberlin's (1890) multiple working hypotheses. Otherwise, the problem of paradigm-shifting will persist over time. I hope this short note can help clarify some of the necessary conceptual tenets to consider regarding the origin of Neotropical diversity and will serve to stimulate the continued constructive discussion on this hot topic, which will enable us to better comprehend our biosphere, its origin and its conservation.

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Figure caption

Figure 1 Schematic view of the six conceptual steps discussed in the text for making inferences about the origin of biodiversity using palaeoecological and molecular phylogenetic evidence.

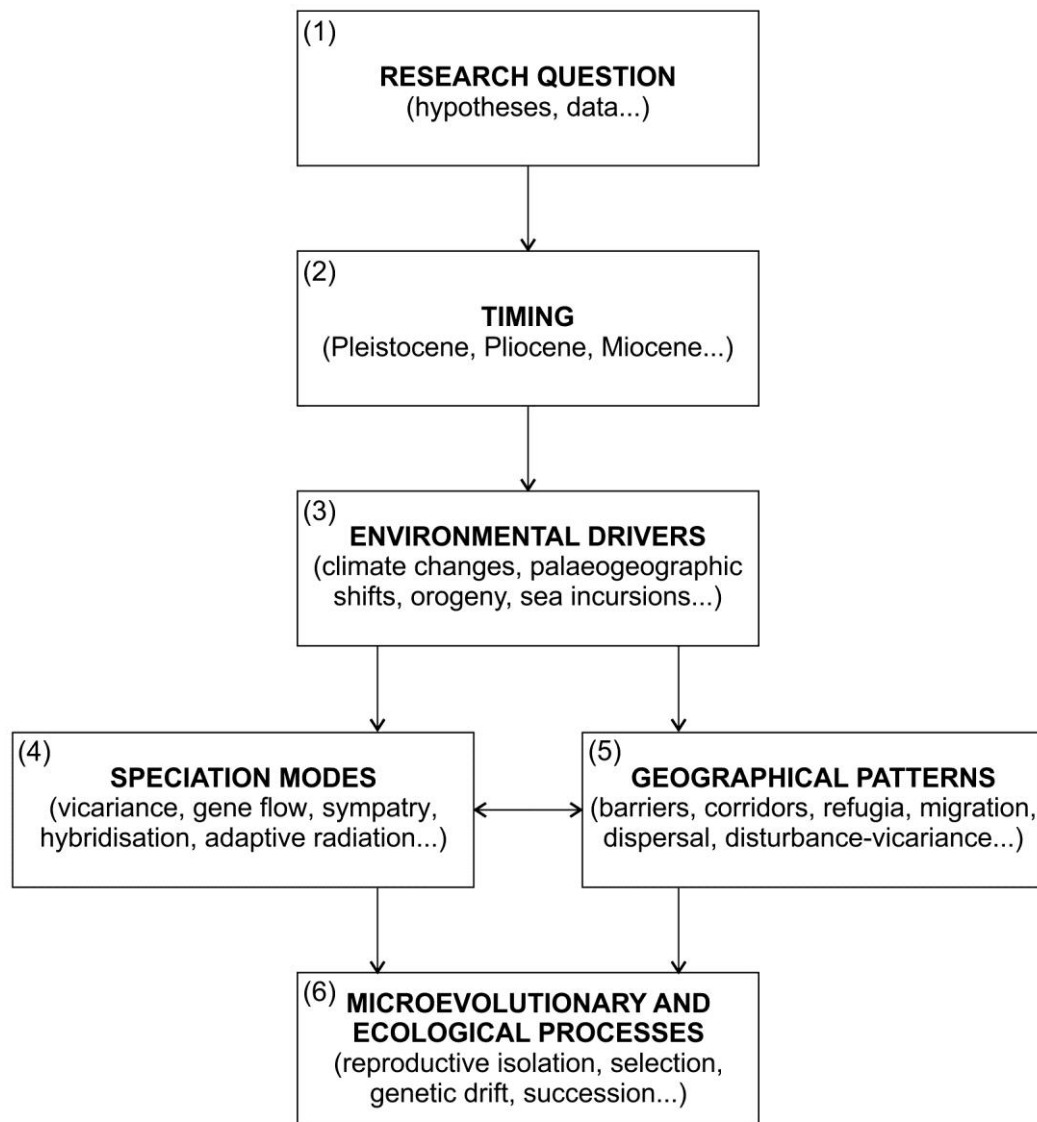


Figure 1